[0052] In selecting for nuclease resistance, it is important not to detract from binding affinity. Certain phosphorus based linkage have been shown to increase nuclease resistance. The above described phosphorothioate linkage increase nuclease resistance, however, it also causes loss of binding affinity. Thus, generally for use in this invention, if phosphorothioate internucleotide linkage are used, other modification will be made to nucleotide units that increase binding affinity to compensate for the decreased affinity contribute by the phosphorothioate linkages.

[0053] Other phosphorus based linkages having increase nuclease resistance that do not detract from binding affinity include 3'-methylene phosphonates and 3'-deoxy-3'-amino-phosphoroamidate linkages. A further class of linkages that contribute nuclease resistance but do not detract from binding affinity are non-phosphate in nature. Preferred among these are methylene(methylimino) linkages, dimethylhydraxino linkages, and amine 3 and amide 4 linkages as described (Freier and Altmann, *Nucleic Acid Research*, **1997**, *25*, 4429-4443).

[0054] There are a number of potential items to consider when designing oligonucleotides having improved binding affinities. It appears that one effective approach to constructing modified oligonucleotides with very high RNA binding affinity is the combination of two or more different types of modifications, each of which contributes favorably to various factors that might be important for binding affinity.

[0055] Freier and Altmann, *Nucleic Acids Research*, (1997) 25:4429-4443, recently published a study on the influence of structural modifications of oligonucleotides on the stability of their duplexes with target RNA. In this study, the authors reviewed a series of oligonucleotides containing more than 200 different modifications that had been synthesized and assessed for their

hybridization affinity and T_m. Sugar modifications studied included substitutions on the 2'-position of the sugar, 3'-substitution, replacement of the 4'-oxygen, the use of bicyclic sugars, and four member ring replacements. Several nucleobase modifications were also studied including substitutions at the 5, or 6 position of thymine, modifications of pyrimidine heterocycle and modifications of the purine heterocycle. Numerous backbone modifications were also investigated including backbones bearing phosphorus, backbones that did not bear a phosphorus atom, and backbones that were neutral.

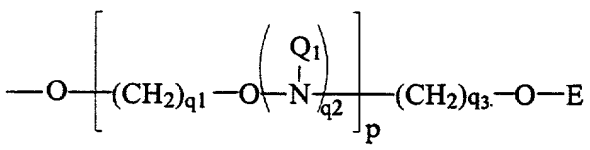
[0056] Four general approaches might be used to improve hybridization of oligonucleotides to RNA targets. These include: preorganization of the sugars and phosphates of the oligodeoxynucleotide strand into conformations favorable for hybrid formation, improving stacking of nucleobases by the addition of polarizable groups to the heterocycle bases of the nucleotides of the oligonucleotide, increasing the number of H-bonds available for A-U pairing, and neutralization of backbone charge to facilitate removing undesirable repulsive interactions. We have found that by utilizing the first of these, preorganization of the sugars and phosphates of the oligodeoxynucleotide strand into conformations favorable for hybrid formation, to be a preferred method to achieve improve binding affinity. It can further be used in combination with the other three approaches.

[0057] Sugars in DNA:RNA hybrid duplexes frequently adopt a C3' endo conformation. Thus modifications that shift the conformational equilibrium of the sugar moieties in the single strand toward this conformation should preorganize the antisense strand for binding to RNA. Of the several sugar modifications that have been reported and studied in the literature, the incorporation

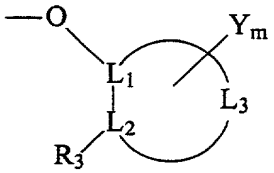
of electronegative substituents such as 2'-fluoro or 2'-alkoxy shift the sugar conformation towards the 3' *endo* (northern) pucker conformation. This preorganizes an oligonucleotide that incorporates such modifications to have an A-form conformational geometry. This A-form conformation results in increased binding affinity of the oligonucleotide to a target RNA strand.

[0058] Representative 2'-substituent groups amenable to the present invention that give A-form conformational properties to the nucleotides include 2'-O-alkyl, 2'-O-substituted alkyl and 2'-fluoro substituent groups. Preferred for the substituent groups are various alkyl and aryl ethers and thioethers, amines and monoalkyl and dialkyl substituted amines. A particular preferred group include those having the formula I or II:

ore



I



II

wherein

E is C_1-C_{10} alkyl, $N(Q_1)(Q_2)$ or $N=C(Q_1)(Q_2)$;

[0059] each Q_1 and Q_2 is, independently, H, C_1-C_{10} alkyl, dialkylaminoalkyl, a nitrogen protecting group, a tethered or untethered conjugate group, a linker to a solid support, or Q_1 and Q_2, together, are joined in a nitrogen protecting group or a ring structure that can include at least one additional heteroatom selected from N and O;

R_3 is OX, SX, or $N(X)_2$;